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ENDOGENOUS LEVELS OF ADENOSINE IN OBESE PATIENTS BEFORE AND AFTER HYPOCALORIC DIET TREATMENT

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ABSTRACT

Obesity is defined as excess body fat and it represents a public health problem in adults and children around the world. It has been suggested that this disease is a chronic inflammatory state, as a result of the enhancement production of inflammatory-related markers, such as adenosine (AD).The objective of this present study was to describe the levels of AD in plasma before and after treatment with a hypocaloric diet as well as anthropometric measurements. Our preliminary data shows that hypocaloric regimen decreased the obesity-related parameters. However, plasma samples containing AD showed no significant changes in obese patients after the treatment of hypocaloric diet. Further studies are needed to evaluate whether AD may be used as a marker for recognizing obesity and effectiveness of treatments.

KEYWORDS: Obesity, Adenosine, Body Fat, Diet, Plasma

INTRODUCTION

Obesity is defined as excessive accumulation of fat in the body and it includes a clinical feature of body mass index (BMI) greater than 30kg/m[2][1] and it has become a public health problem in several countries, including the United States of America. [2]This disturbance causes health problems such as heart diseases, hypertension, type 2 diabetes, cancer, etc.[3, 4] The etiology of obesity is complex and involves different elements in its installation and development, including factors such as socioeconomic status, inheritance, cultural aspects, life style, among others.[5, 6] Moreover, obesity is recognized as a chronic inflammatory disease.[7] From the biochemical point of view there are different molecules related to obesity, including proteins, lipids, and neurotransmitters.[8, 9] Recently it has been described that a purine, such as adenosine (AD), is also involved in obesity. Regarding this issue, several studies have reported suggesting the role of AD in

inflammatory processes associated with obesity.[10, 11, 12] In this regard, Escudero et al. (2012) reported high levels of AD in plasma in obese children[13], while Jadhav and Jain (2012) demonstrated that AD deaminase levels were increased in obese subjects.[14] Taking together this data, it was reasonable to hypothesize that AD levels in obese subjects would be decreased after a hypocaloric diet as well as anthopometric measurements. Thus, the aim of the present study was to compare the levels of AD in plasma before and after treatment with a hypocaloric diet as well as anthropometric measurements.

METHODS

Bioethical Considerations

The experimental protocol was approved by the Committee for Research and Bioethics of the School of Medicine of our University. During the development of the project, we ensured that the use of personal information was strictly for the purposes of the analysis of data whereas the management of biological samples followed the Guidelines of the Official Mexican Norm NOM-253-SSA1-2012 as well as the Guidelines of Hazardous Waste Management Biological-Infectious (derived from the Mexican Official Norm [NOM-087-ECOL-SSA1-200]). This study was conducted in accordance with the World Medical Association (Declaration of Helsinki) and all the subjects that participated in the research gave their written informed consent for inclusion before they participated in the study. The data were analyzed anonymously throughout the study.

Materials And Reagents

Blood collection tubes with anticoagulant (EDTA) and isopropyl ethyl alcohol 70% were obtained from Sigma Chemicals (St. Louis, MO. USA). Needles 0.9mm to 1.1mm in diameter (20g-19g; Terumo. Japan), adapter-pipes Vacutainer (BD Diagnostics, NJ. USA), cotton, gloves, adhesive bandages, and band aids were used. For detection and quantification of AD using the High-Performance Liquid Chromatography (HPLC), reagents were acquired from Sigma Chemicals (St. Louis, MO. USA).

Subjects

A total of 13 apparently healthy adult Mexican men(n=5) and women (n= 8) from Metropolitan area of Mérida City that attended outpatient Nutrition Clinic in the Clínica Universitaria Anáhuac Mayab located in Mérida, Yucatán (México) were enrolled in the study. All of the subjects signed the informed conset before the studies. Patients were excluded from the study if they had previous or recent diagnosis of cancer, endocrine disorders, blood pressure higher than 140/90mm Hg, diabetes mellitus, or inflammatory or autoimmune disorders. Subjects under anti-hypertensive drugs, cardiometabolic medication (including anti-inflammatory drugs), as well as subjects without having an 8-12h overnight fasting were excluded from the study. Female subjects were excluded if pregnant or lactating condition was present at the beginning of the study. Physician examined all subjects that were enrolled in the report by receiving medical and physical evaluation.

Interventions

After signing informed conset, subjects were diagnosed in Session 1 as obese according to the World Health Organization criteria for Body Mass Index (BMI).[1] Then, they were appointed for a second consult (Session 2) at 10:00h in the Nutrition Clinicfor baseline of anthropometric measurements (see section *2.5 Anthropometric Measurements*), and biochemical sampling (blood samples collection; see section *2.6 Blood sampling collection*). Next, in Session 2 the estimation of total energy expenditure was determined (see section *2.7 Estimation of the Total energy expenditure)* and a

hypocaloric diet was given to the patients (see section *2.8 Hypocaloric diet*). All data collected from Session 2 were considered as pre-treatment condition.Thirty days later, patients were appointed for Session 3 at 10:00h to obtain anthropometric measurements as well as blood samples collection. The data obtained from Session 3 were considered as post-treatment condition.

Anthropometric Measurements

Anthropometric measurements were carried out at 10:00h at Nutrition Clinic. The BMI was obtained in obese patients (BMI $\geq$30 Kg/m^2) by dividing weight by height squared (Kg/m2) whereas waist circumference was obtained using standard procedures such as considering the midpoint between the lower rib margin and the iliac crest, and it was measured by using a metalic tape in centimeters (cm; LUFKIN, w606PM. Australia). The varibles such as BMI (Kg/m^2), fat (%), TBW (Kg), fat mass (Kg), trunk fat mass (Kg) were calculated by using a body composition analyzer (INBODY®, Body Composition Analyzer, model Inbody 720. Korea, Japan). The procedures used in this section of the study met the internationally accepted criteria.[15] The antopometric measurements procedure werecarried out before and after the hypocaloric diet.

Blood Sampling Collection

Following the anthropometric measurements, patients were placed in a chair for blood collection (8mL) into pyrogen-free tubes (Vacutainer TM, BD Diagnostics, NJ. USA) containing EDTA anticoagulant (Sigma Chemicals; St. Louis, MO. USA). Collection tubes were centrifuged at 1000g/4°C for 30min, and serum samples were obtained and stored at −80◦C until further AD analysis. This procedure was developed before and after the hypocaloric diet treatment.

Estimation of the Total Energy Expenditure (TEE)

For diet composition, the estimation of TEE was determined by the number of Kcal that subjects consumed by using the indirect calorimetric measurement (KORR, Reevue. Salt Kale, UT. USA), which estimates the rest energy expendidure (REE). This procedure was used as previously reported by other groups.[16]

Hypocaloric Diet

At the end of Session 2, dietary guidance was provided to patients. The treatment consisted in a hypocaloric diet (reduction of 500 kcal of total diet) with a distribution of macronutrients as follows: Carbohydrates 50%, protein 22% and fat 28%. The hypocaloric diet was based on previous reports.[17, 18] During the time between Session 2 and Session 3, reminders of the diet were made to patients through phone calls once a week.

Determination of Adenosine Levels by HPLC

Blood samples collected before or after hypocaloric diet treatment were processed for analysis of the levels of AD using the HPLC (Modular Prominence HPLC, Shimadzu. Japan). Separation was using a C18 column (5µm, 50 x 4.6mm; Shimadzu. Japan) within temperature controlled (22ºC; oven CTO-20A. Shimadzu. Japan). Detection of AD was carried out by using an UV detector (254nm; SPD-20A Prominence. Shimadzu. Japan) coupled to HPLC. The mobile phase consisted of 10mM sodium dihydrogen phosphate (pH 4.5) and methanol (9%) and it was infused at a flow rate of 80µL/min using a pump (LC-20AT. Prominence HPLC, Shimadzu. Japan). Once the samples were automatically injected (SIL-20A HT Prominence HPLC, Shimadzu. Japan) into the HPLC,the chromatographic data was stored on a personal computer (via computer controller. CBM-20A. Shimadzu. Japan) and concentrations of AD were calculated using the

software HPLC (LC LabSolution. Shimadzu. Japan) comparing the sample peaks with known standards. The procedures for analysis of the concentrations of AD were carried outas previously published our group.[19]

Statistical Analysis

The results are represented as mean ± standard error of the mean. To determine the differences between anthropometric values in obese subjects (before vs. aftertreatment of hypocaloric diet) it was used a Student *t* test with a significance level of $P<0.05$. For comparisons between AD plasma levels in obese subjects (before vs. after hypocaloric diet) data wereanalyzed using a Student *t* test with a significance level of $P<0.05$. Finally, to determine the correlations between anthropometric measurements and levels of AD (before or after intervention) a Pearson correlation coefficient was used. Acceptance of the index value waspositive if correlation was $0 < r < 1$. All statistical analyses were determined using StatView (version 5.0.0; SAS Institute. USA).

RESULTS

In accordance with previous results[17, 18] obese patients treated with hypocaloric diet decreased the anthropometric measurements of TBW (Kg), BMI (Kg/m^2), fat (%), fat mass (Kg), visceral fat area (cm^2), trunk fat mass (Kg), and waist circumference (cm$^{2;}$ Table 1).

	Pre-diet	**Post-diet**	***P*-value**
Age	41.61 ± 13.2	41.61 ± 13.2	N.S.
n/Women)	5/8	5/8	N.S.
Total body weight (Kg)	88.70 ± 3.86	86.85 ± 3.95	P <0.0001
Body mass index (Kg/m^2)	35.07 ± 0.97	34.30 ± 1.01	P <0.0004
Fat (%)	44.10 ± 1.70	42.78 ± 1.84	P <0.0003
Fat Mass (Kg)	38.90 ± 1.9	36. 96 ± 2.0	P <0.0001
visceral fat area (cm^2)	145.53 ± 6.5	139.9 ± 6.6	P <0.0004
Trunk fat mass (Kg)	20.09 ± 0.8	19.27 ± 0.9	P <0.0002
Waist circumference (cm^2)	112.91 ± 3.0	109.73 ± 3.2	P <0.0009

Table 1: Anthropometric Parameters in Obese Subjects Before and After the Treatment of Hypocaloric Diet during 30 Days (Data are Shown as Mean ± S.E.M. *p < 0.05)

Plasma samples containing AD (pg/mL) were assessed in obese patients before and after the treatment of hypocaloric diet. In our study, AD was found not statistically different among pre- and post-diet sessions (Figure 1, Panel A). Further results showed that only TBW and AD contents before diet were negatively and significant correlated (r= -0.645, $p<0.01$; Figure 1, Panel B). A positive correlation with statistical significance was observed among Fat (%) and

AD levels before the hypocaloric diet (r= 0.606, p <0.02; Figure 1, Panel C). No significant correlations were found among BMI, fat mass, vicseral fat area, trunk fat mass or waist circumference and AD levels pre- and post-diet (data not show).

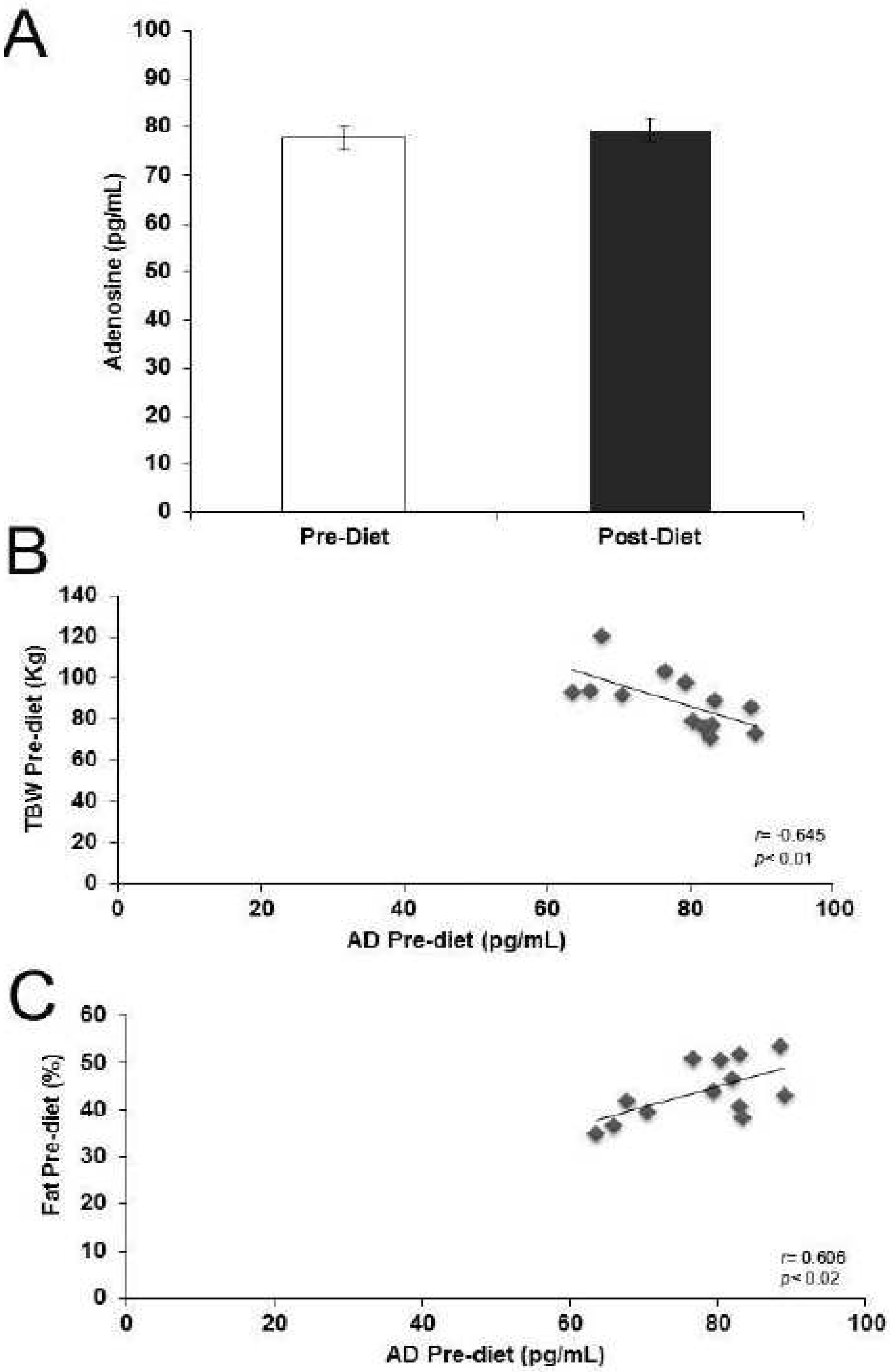

Figure 1: Adenosine levels in plasma (pg/mL) of obese subjects before and after the treatment of hypocaloric diet during 30 days (Data are shown as Mean ± S.E.M. *p < 0.05; Panel A). Correlation between total body weight (TBW; Kg) and AD plasma levels (pg/mL) pre-treatment (Panel B; *p < 0.05) and correlation among fat (%) and AD contents in plasma (pg/mL) pre-treatment (Panel C; *p < 0.05).

CONCLUSIONS

It is well accepted that obesity can be viewed as inflammatory disorder.[20] Moreover, systemic inflammatory biomarker have been associated with obesity, such as leukotrienes, leptin, tumor necrosis factor alpha among other compunds [21-23] and recently with AD.[13, 24] In this regard, Escudero et al. (2012) reported that AD was detectable in obese children and contents of this molecule were significantly higher compared to non-obese children. [13]However, it is unknown if a treatment to obese subjects would decrease the endogenous levels of AD. Thus, the goal of the present study was to assess whether obese patients subjected to a hypocaloric diet during 30 days would induce a significant modification in AD plasma levels.

In the current report, the obesity-related anthropometric parameters showed a significant decrease with the intervention. However, and in opposition to the hypothesis, AD contents showed no statistically significant changes after the hypocaloric diet in obese subjects. It is important to mention that our preliminary study shows several limitations, such as the 30-days of the treatment, which could representa short period of time to evaluate the impact of a hypocaloric diet. Indeed, it is feasible to expect that plasma contents of AD may be diminished in obese individuals after long-term treatment.

An interesting result is the correlation among TBW and AD levels before the diet as well as fat and AD contents but not after the treatment. Despite that our report did not evaluate mechanisms of action, a possible explanation to understand these apparently controversial findings could be drawn. For example, a recent study published by Chielli et. al. (2014) reported that AD deaminase in obese young subjects is higher in salivary samples compared to non-obese individuals. Therefore, AD deaminase should be also considered for monitoring after diet sincethis enzyme is increased in obese patients.[25, 26]

The role of the adenosinergic system in obesity is still poorly understood. For this reason, it is important to mention that the present preliminary report contributes to the knowledgement of the relevance of determine if diet would decrease levels of AD as an endogenous marker of effectiveness of the treatment. Collectively, these findings suggest that despite in the improvement in obesity-related parameters, AD levels showed no statistical changes after intervention. Whether AD diminution in these patients would represent a significant improvement in health is a matter worthy to be considered in future clinical studies.

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STATMENT OF AUTHORSHIP

All authors listed in the MS have made significant contributions to the study.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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